EFFECT OF X-RADIATION ON THE DESOXYRIBONUCLEIC ACID AND ON THE SIZE OF GRASSHOPPER EMBRYONIC NUCLEI [1,2]

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It is now believed that the vital activities of cells are controlled to a great extent by nucleoproteins. Only a beginning has been made toward attaining an understanding of the biological importance of nucleic acids. Research methods which involve the use of agents that disturb the normal balance of the nucleic acids are being utilized, and by these means it should be possible to reach some definite conclusions on the role played by the nucleic acids in living cells.

Since desoxyribonucleic acid (DNA) is a universal constituent of chromatin, it probably has a significant part in inheritance, in regulating cell division, and perhaps in the formation of proteins and of ribonucleic acids of the nucleolus and cytoplasm (Caspersson, 1936, 1940, 1941; Caspersson and Schultz, 1938, 1940). Since x-rays produce mutations and chromosomal aberrations, observations on their effect on the DNA content of the cells are of considerable importance. We had previously noted in this laboratory that irradiated nuclei seem to stain less intensively with the Feulgen reagent than non-irradiated nuclei. The purpose of the present study was to determine by photometric methods, which make possible an accurate quantitative cytochemical study of the nucleoproteins, whether there is an actual change in the quantity of DNA after irradiation. Examination of our photometric data sheets disclosed that the diameters of irradiated nuclei were usually larger than those of control nuclei. This fact, along with an apparent increase in size observed in irradiated neuroblasts in living culture preparations, led to a study of the effect of radiation on nuclear size.

Methyl green stain and the Feulgen reaction were used for the DNA determinations. With these two stains it is possible to analyze for two different properties of the nucleic acid. The Feulgen is specific for the DNA desoxypentose content and the methyl green probably indicates the presence of DNA in the normal state of polymerization, commonly called the "highly polymerized state." Most workers now agree that, when the nuclear reaction takes place under properly controlled conditions, the Feulgen method gives an accurate relative estimate of the DNA in nuclei (Hillary, 1940; Baker, 1942; Stowell, 1945, 1946; Di Stefano, 1948). Ultraviolet absorption studies of Wyckoff, Ebeling and Ter Louw (1932) identify the Feulgen-positive material in the nucleus with DNA. Also, splenic and pancreatic desoxy-

[1] Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

[2] For work reported in this paper the authors were awarded the research prize of the Association of Southeastern Biologists at the 1951 annual meeting in Tuscaloosa, Alabama.

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ribonucleases remove the Feulgen-positive bands (presumed to contain large amounts of DNA) from the salivary gland chromosomes of Drosophila (Catcheside and Holmes, 1947). Although there is evidence that the reaction does not indicate the total amount of DNA present (Caspersson, 1932; Ely and Ross, 1949), many workers have shown it is valid to use the Feulgen reaction with the photometric system to determine the relative amounts of DNA in nuclei (Ris and Mirsky, 1949; Lumb, 1950; Leuchtenberger, 1950; Swift, 1950). The specificity of methyl green for polymerized DNA was demonstrated by Pollister and Leuchtenberger (1949), Leuchtenberger (1950), and Kurnick (1950a, 1950b, 1950c). Leuchtenberger found Feulgen and methyl green extinction ratios to be equal in normal nuclei.

Material and Methods

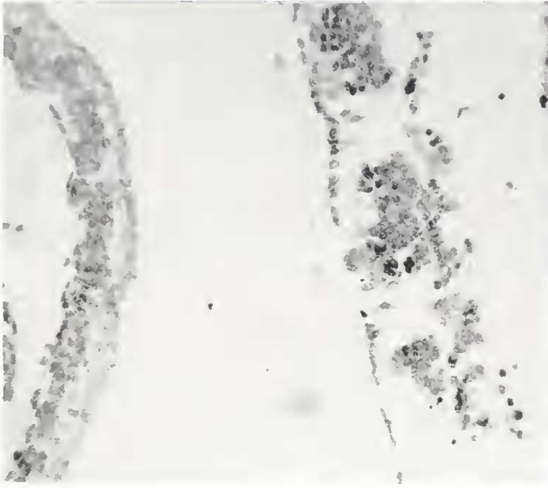
The present cytochemical studies were made with 14-day-old embryos of the grasshopper, *Chortophaga viridifasciata* (De Geer). The control and irradiated embryos for each experiment were from the same egg case. Doses of 4000, 10,000, and 12,500 r of x-rays were given. The embryos were fixed 10 hours after irradiation, since the maximum visible difference between the irradiated and control tissue, along with the maximum swelling of the irradiated nuclei, was observable at this time (Figs. 1–3). Both control and irradiated eggs were kept at 38° C. between the time of irradiation and fixation. Treatment of the control and x-rayed embryos was identical throughout each experiment except for the irradiation, thereby ruling out any disparity of the results from other causes and making valid a comparison of the cells of the irradiated and control embryos.

Since many cytologists (Koller, 1947; Darlington and Mather, 1949; Serra, 1949; etc.) believe that the content of DNA per nucleus varies with the different stages of mitosis, all photometric measurements were made on cells in the mitotic stages in which the chromatin is most widely dispersed, namely, late telophase, interphase, and early prophase. All three of these stages were used because it was difficult to distinguish them from one another in material which had been treated by immersion in approximately 20 per cent of the isotonic salt concentration to make the chromatin more uniformly distributed throughout the nuclei.

Two different x-ray units were used in these studies. The first experiments were done with a Coolidge Universal x-ray tube, operating at 90 kilovolts and 5 milliamperes. During exposure the material was 18.4 cm. from the center of the target, and the long wave length x-rays were removed by a 0.3 mm. aluminum filter. The intensity was about 250 r per minute. Later, when the previous experiments were repeated and additional studies made, irradiation was carried out with a General Electric Maximar 250 kv unit operating at 250 kilovolts and 15 milliamperes. An aluminum filter of 0.5 mm. was added to the 3 mm. aluminum inherent filtration of the machine. When the material was 54 cm. from the center of the target, the intensity was about 250 r per minute. Monitoring with a Victoreen thimble chamber preceded each x-ray treatment.

All material to be sectioned was fixed with Carothers fluid.[5] The fixation time

[5] 75 cc. of a saturated aqueous solution of picric acid; 15 cc. of formalin, C. P.; 10 cc. of glacial acetic acid; 1 gm. urea crystals. Warm slightly and stir thoroughly as the urea is added. Add 4 drops of a 50 per cent aqueous solution of chromic acid to 5 cc. of the above solution just before using.



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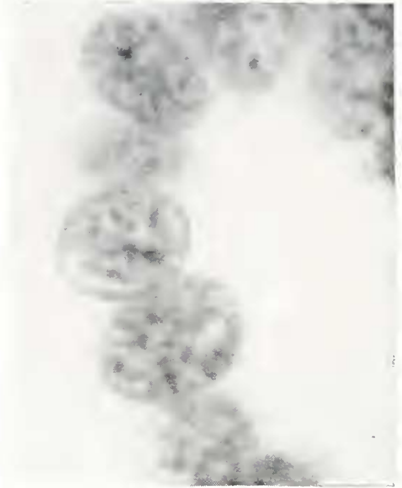

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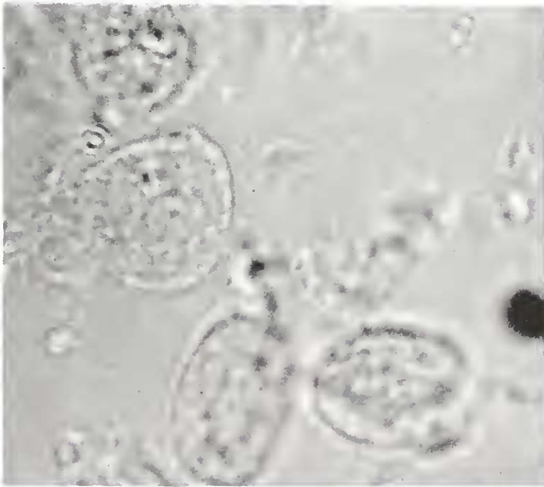

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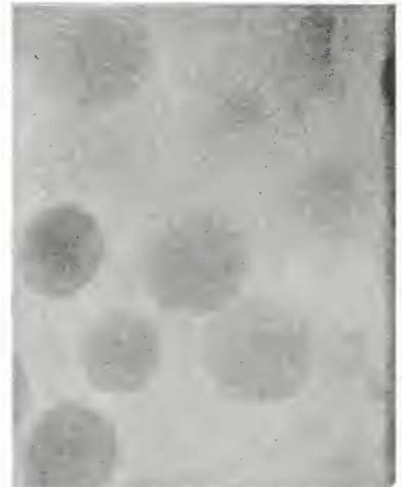

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FIGURE 1. Note the difference in Feulgen stainability between the irradiated embryo (4000 r) on the left and the non-irradiated embryo on the right. Fixed with Carothers fluid 10 hours after treatment. 100 ×.

FIGURE 2. Heterogeneous un-irradiated nuclei. Carothers fixation. Feulgen stained. 900 ×.

FIGURE 3. Heterogeneous irradiated nuclei. Fixed with 4 per cent neutral formalin, stained with methyl green. Note the enlarged nuclei. 900 ×.

FIGURE 4. Un-irradiated nuclei pre-treated with a hypotonic solution that made the chromatin more uniformly distributed. Fixed in 4 per cent neutral formalin, stained with methyl green. 900 ×.

of the control and treated embryos for each experiment was the same, varying from 20 to 24 hours for the different experiments. The embryos were washed in distilled water for one hour and then prepared for sectioning by dehydrating with ethanol. The picric acid of the fixative was removed in a 70 per cent ethanol solution saturated with lithium carbonate. After dehydration was complete, the material was cleared in aniline oil and chloroform and embedded in Tissuemat. The control embryo and treated embryo were embedded side by side in the paraffin block and sectioned simultaneously. Sections were cut at 16 microns, then decerated and hydrated. A hydrolysis curve for each time was run in normal HCl (58° C.) for 10, 20, 30, and 40 minutes. The optimum Feulgen staining reaction, which was used for photometric determinations, was obtained after 20-minute hydrolysis. The Feulgen stain was prepared as specified by Coleman (1938). With the exception of the staining time, 3 hours for these sections, the staining schedule of Darlington and LaCour (1947) was followed.

Embryos dissected in a hypotonic solution and then fixed in acetic-alcohol or neutral formalin have nuclei which show a more uniform distribution of chromatin than cells dissected in an isotonic solution (Figs. 2, 3, 4). Variations in the absorption measurements of a nucleus were reduced by making the chromatin more uniformly distributed. Smears of the more uniform-appearing nuclei were stained with Feulgen and methyl green in the following manner. Embryos were removed from the eggs and dissected in a hypotonic culture medium and left in the solution for 10 minutes. Cells that were to be stained with the Feulgen reagent were fixed in Carnoy fluid (3 : 1) for 30 minutes. After hydrolysis for 10 minutes in normal HCl at 60° C., the embryos were rinsed in distilled water, stained with leucobasic fuchsin for 15 minutes, softened for smearing by treating with 45 per cent acetic acid for 1 minute, and then mounted and smeared in a Karo-formalin solution (1 part Karo, 1 part formalin, 3 parts water). The preparation and methyl green staining procedure described by Kurnick (1950a) was followed, with the exceptions that the embryos were dissected in a hypotonic culture medium before fixation and that the staining was done at 17° C. Cells treated in the same way except for staining were used to determine the blank, *i.e.*, the absorption of unstained nuclei. Correction for non-specific light loss was not made after it was shown that unstained nuclei are practically invisible and transmit from 93 to 96 per cent of the light.

Measurements of nuclear diameters were made with an oil immersion objective (90 ×) and a 10 × ocular which contained a calibrated micrometer disk. Since all the nuclei were not exactly spherical, four different diameters of each nucleus were read and averaged. The mean of ten such averages, all on nuclei from the same embryo, was obtained for each dose of x-rays and for the corresponding controls. Over 300 nuclei were measured. An example of the data is shown in Table I.

The photometric apparatus used is similar to that described by Swift (1950) with the micro-ammeter replaced by an amplifier and Brown recorder. The recorder was originally installed to facilitate some tests of the apparatus, and since it offered the advantage of an automatic permanent record of the photoelectric current, it was not removed from the system. The DNA stained with the Feulgen reagent was measured by the absorption of green light isolated from a Spencer microscope lamp by means of Corning Glass filters No. 3484, No. 4010, and No. 4303. The DNA stained with methyl green was measured in terms of the absorption of red light iso-

lated by Corning Glass filter No. 2408. Optics were an A. O. 50 × reflecting objective and a Zeiss 10 × ocular.

DNA content is expressed in relative instead of absolute units, because several assumptions are made with photometric microscopic measurements, namely, that the nuclei are perfect spheres, and that the material is homogeneously distributed throughout the nucleus. With Feulgen-stained material variations in fixation and staining procedure may produce variations in the intensity of the reaction. Also, potentially stainable material may be lost during hydrolysis. In view of these assumptions and possible sources of error, it is not feasible to determine absolute amounts of DNA per nucleus. All values of DNA reported are, therefore, in arbitrary units.

TABLE I

Data showing measurements of diameters of control and irradiated (4000 r) nuclei. Sections fixed with Carothers fluid, hydrolyzed 20 minutes, and Feulgen-stained

Embryo	Nuclei	Diameter measurements				Average	Mean
		1	2	3	4		
Irradiated (4000 r)	1	10.5	10.0	11.0	10.5	10.50	
	2	10.5	10.0	8.5	8.0	9.25	
	3	9.0	9.0	8.5	8.0	8.62	
	4	8.5	8.5	7.5	9.0	8.37	
	5	10.5	10.0	11.0	11.0	10.62	
	6	8.0	8.5	8.5	8.0	8.25	
	7	8.5	10.5	10.0	10.5	9.87	
	8	9.0	10.0	9.0	11.0	9.75	
	9	8.0	9.0	8.0	9.0	8.50	
	10	9.0	8.5	8.5	8.5	8.62	
							9.24
Control	1	8.5	10.0	10.0	10.5	9.75	
	2	7.5	8.0	8.5	7.5	7.87	
	3	7.5	8.0	8.5	7.5	7.87	
	4	6.5	7.5	7.5	7.5	7.25	
	5	8.5	8.0	6.0	7.5	7.50	
	6	8.0	8.5	8.0	8.0	8.12	
	7	9.0	9.0	8.0	9.0	8.75	
	8	8.5	8.0	10.0	9.0	8.87	
	9	9.0	8.5	9.0	10.0	9.12	
	10	9.0	8.5	9.0	10.5	9.25	
							8.43

A brief explanation of the photometric routine will aid in understanding the tables. A nucleus which is to be measured is centered in the microscope field and brought into sharp focus. The photometric apparatus is adjusted over the ocular, and an enlarged image of the nucleus is observed through a magnifying lens placed immediately above the field diaphragm of the photometer. Measurements of the nuclear diameter are made, after the nucleus has been made concentric with the micrometer disk in the image plane. An aperture of 1, 2, or 3 millimeter diameter is

inserted in the image plane, so that only a central core of the nucleus is visible. The magnifier is then swung out, and the phototube is moved into place over the aperture. Light from the central core of the nucleus now falls on the photosensitive cathode, and a reading is made with the Brown recorder. Immediately the slide is moved

TABLE II

Samples of raw data, showing the calculations made in obtaining the arbitrary units given in subsequent tables

Nuclear diameter* (μ)	I_n	I_0	I_0/I_n (1/Transmission)	Extinction (log $1/T$)	$1/F$	EC^2 $(1/F)$
Nuclei irradiated 4000 r. Carothers fixation. Feulgen sections.						
9.2	59.1	93.2	1.577			
	59.6	92.7	1.555			
	59.6	92.4	1.550			
	60.7	91.7	1.511			
	58.8	92.4	1.571			
			1.553	0.19117	6.476	2.78
9.0	53.7	91.8	1.709			
	52.8	93.1	1.763			
	53.3	91.1	1.709			
	54.8	91.6	1.671			
	52.5	90.6	1.726			
			1.716	0.23452	6.115	3.23
Non-irradiated (control) nuclei. Carothers fixation. Feulgen sections.						
6.5	40.8	91.8	2.250			
	40.4	90.9	2.250			
	40.5	92.4	2.281			
	40.4	90.7	2.245			
	40.2	90.3	2.246			
			2.254	0.35295	3.29	2.61
6.2	30.1	86.6	2.877			
	29.1	87.1	2.993			
	29.0	87.6	3.021			
	29.8	87.2	2.926			
	30.6	87.4	2.856			
			2.935	0.46761	2.99	3.15

* An average of five measurements.

and a clear area of the slide is measured in a similar manner. Two readings, the first through the nucleus and the second through the background, are made for each determination. The intensity of the light passing through the clear area (I_o) divided by the intensity of the light transmitted by the nucleus (I_n) gives the reciprocal

of the transmission ($1/T$). Transmission data are then converted into extinction values (E):

$$E = \text{Log}_{10}\, 1/T.$$

This extinction value represents the light transmission for the central core of the nucleus and not through the entire nucleus. A value which gives the relationship of the area measured to the entire volume of the cell is $C^2\, 1/F$.

$$1/F = \frac{R^3}{R^3 - (R^2 - C^2)^{3/2}}$$

C is the radius of the cylinder or the radius of the aperture used to make the reading. R is the radius of the spherical nucleus. Then $EC^2 1/F$ = extinction value for the total volume of the nucleus (Table II). Further details of the computations and corrections for photometric measurements are discussed by Swift (1950), Pollister and Ris (1947), and Di Stefano (1948).

Statistical Treatment

To ascertain whether a significant difference existed between the mean diameters of irradiated and control nuclei, a test devised by Mosteller (1948) was used. The 2.0 per cent level was accepted as significant.

A two-way analysis of variance table was used to determine the difference in DNA (Feulgen and methyl green) content of irradiated and control nuclei. Unless stated otherwise, the 5 per cent probability level was accepted as the significant value.

Results

Nuclear diameter. When studying a series of Feulgen-stained nuclei of any one type, the fact becomes apparent that smaller nuclei are more darkly stained than larger nuclei. This condition was also noted by Swift (1950). Measurements of the diameters of nuclei of the Feulgen and methyl green material showed that the irradiated nuclei had significantly larger diameters than the non-irradiated (Tables III

Table III

Data showing mean diameters of control and irradiated nuclei. Feulgen-stained section; Carothers fixation

Treatment	Hydrolysis time (min.)	Number of nuclei measured	Mean diameter (μ)
X-ray 4000 r	10	20	11.55
Control	10	20	10.29
X-ray 4000 r	20	40	11.85
Control	20	40	10.85
X-ray 4000 r	30	20	11.70
Control	30	20	10.22
X-ray 4000 r	40	20	11.50
Control	40	20	10.42

TABLE IV

Data showing mean diameters of control and irradiated nuclei. Methyl green smears; fixation in 4 per cent neutral formalin

Treatment	Number of nuclei measured	Mean diameter (μ)
X-ray 4000 r	30	10.13
Control	30	8.26
X-ray 10,000 r	20	11.15
Control	20	9.47

and IV). Nuclei that had received an x-ray dose of 12,500 r were not significantly larger than nuclei receiving only 4000 r. Therefore, the degree of swelling at 10 hours after treatment does not appear to be related to the dose of irradiation received. Living-culture experiments performed in this laboratory have shown that the neuroblasts of grasshopper embryos swell after receiving any dose greater than 100 r of gamma or x-radiation. This swelling is independent of the dose rate, as it was observed whether a dose of 100 r was given in 1 minute or in six days. A change in osmotic pressure accompanies the swelling of cells, since after irradiation the cells are hypertonic in a culture medium in which they were isotonic previous to irradiation.

TABLE V

*Average amounts of DNA per nucleus in embryonic grasshopper tissue, as obtained by photometric determinations of Feulgen preparations **

	Arbitrary units of DNA			
	Carothers fixation (sectioned material)		Carnoy fixation (smears)	
	Control	Irradiated 4000 r	Control	Irradiated 12,500 r
	3.18	2.78	3.51	4.93
	2.80	3.23	2.99	4.99
	2.61	2.61	3.55	4.90
	6.22†	2.34	3.72	3.94
	3.15	2.19	2.86	2.48
	3.05	2.83	3.15	3.02
	3.16	2.89	3.27	3.44
	3.32	2.20	3.62	2.96
	2.91	3.32	3.92	3.28
	2.96	2.38	2.72	4.83
	3.00	2.87	3.08	4.52
	2.69	3.00	2.89	4.86
	2.73	3.23	3.59	2.83
	3.42	3.18	2.27	3.84
	3.23	2.22	3.42	3.69
	2.69			
Mean	2.99	2.75	3.24	3.90
Standard error	0.28	0.26	0.31	0.40

* Each value is an average of five readings on one nucleus.
† Not averaged with other values since this cell probably had 4N DNA content.

Feulgen determinations. Photometric measurements on tissue stained with Feulgen show, when corrections for nuclear volume are made, that approximately the same number of dye molecules exist in irradiated and non-irradiated nuclei. Statistically, there was no significant difference between the irradiated and control cells (Table V).

Methyl green determinations. Photometric data on methyl green stained material revealed a highly significant difference (1 per cent level) between the extinction values of the irradiated (4000 r and 10,000 r) and non-irradiated nuclei. The results are summarized in Table VI.

Table VI

Average amounts of DNA per nucleus in embryonic grasshopper tissue, as obtained by photometric determinations of methyl green preparations *

	Arbitrary units of DNA			
	Control	Irradiated 4000 r	Control	Irradiated 10,000 r
	6.34	3.11	4.45	2.66
	5.92	2.44	2.29	1.90
	4.23	2.13	5.66	1.89
	4.86	1.79	6.74	1.45
	4.57	1.85	3.48	0.88
	6.02	2.55	5.83	1.46
	5.36	2.02	5.22	1.78
	5.86	2.43	3.46	1.83
	5.55	3.01	2.99	1.48
	6.00	3.02	6.42	1.96
	4.23	1.87	3.25	1.83
	4.86	1.58	3.48	2.02
	4.43	2.31	2.92	2.42
	5.57	2.46	4.54	2.23
	4.69	2.98	6.83	2.41
	4.36	2.72	6.47	1.87
	4.15	2.83	5.25	2.36
	6.55	1.89	5.83	1.84
Mean	5.20	2.39	4.73	1.90
Standard error	0.19	0.11	0.35	0.10

* Each value is an average of five readings on one nucleus.

With nuclei which had been made fairly homogeneous, it was difficult to distinguish between late telophase, interphase, and early prophase. Photometric measurements disclosed a few nuclei which had twice the DNA content of the other nuclei (Table V). The increased amount of DNA may be attributed to a polyploid nucleus resulting from a radiation-induced suppression of cytokinesis.

Discussion

The present study has shown that an increase in nuclear size occurs after x-radiation. This has been observed in a wide variety of biological materials. Hol-

weck and Lacassagne (1930) found that yeast spores divided and then swelled after irradiation. Similar results were obtained by Robertson (1935) with protozoa; Lea, Haines and Coulson (1937) with bacteria; Mottram (1927) with mice tumors; Glücksmann (1941) with human tumors. Lea (1947) explained such cases, where the swelling did not appear for several hours or days after treatment, by stating that the effect was due to an inhibition of cellular division while growth continued at a normal rate. If our studies had been based entirely on stained material (fixed 10 hours after irradiation), Lea's explanation would have satisfied the data. However, our experiments with living tissue cultures have shown that swelling occurs in a matter of minutes after treatment. When the larger doses are given, the swelling is instantaneous. A normal growth rate can hardly account for such rapid swelling. Observations on irradiated tissue cultures lead us to believe that water is being absorbed rapidly by the cells, presumably as a result of an x-ray-induced osmotic pressure increase. Failla (1940) suggested that radiation might change the cellular constituents so that the osmotic pressure is increased without altering the permeability of the cellular membrane. The depolymerization or molecular rearrangements of DNA, indicated by the methyl green results, might then be a factor contributing to the increase of osmotic pressure and thus cause the nuclei to swell.

Probably the first study reporting that radiation could increase the nuclear volume without decreasing the amount of chromatin was made by Mottram in 1933 on work with bean root tips. After accurate and detailed visual observations he wrote (p. 218) that the increase in size of the nuclei after radiation "is due to a great increase in the nucleoplasm, the other constituents of the nucleus remaining normal in amount. The chromatin becomes thus more dispersed and has the appearance of being diminished in amount, but in reality it remains normal in quantity." Results suggesting that the nuclear volume may be changed considerably without a significant variation in the Feulgen-stainability of DNA, are in agreement with recent work by Schrader and Leuchtenberger (1950), and Leuchtenberger (1950). The former authors reported that the DNA per nucleus remained constant, despite an eight-fold increase of nuclear volume in the spermatocytes of *Arvelius albopunctatus*. Additional studies disclosed that an increase or decrease of nuclear volume is due mainly to a corresponding modification in the protein, RNA, and water content of the cell and not to an alteration of DNA. Leuchtenberger found in early pycnosis of tumor cells, a condition in which marked reduction of nuclear volume occurred, the Feulgen-stainability of DNA remaining unchanged in quantity. The conclusion seems justified that x-radiation may augment the nuclear volume without destroying the DNA desoxypentose.

The cytochemical studies using methyl green show that some physical property of DNA is affected. Probably depolymerization or some change of the molecular configuration has taken place, and the change is too slight to detect visually. Many investigators report that depolymerization of the DNA may occur after treatment with ionizing radiations. In 1939 Svedberg and Brohult suggested that, directly or indirectly, radiation splits the molecular constituents within the cell. The stickiness and loss of spiralization noted in chromosomes after irradiation may be produced by the depolymerization of nucleic acids (Darlington and La Cour, 1945). Sparrow and Rosenfeld (1946), and Taylor, Greenstein and Hollaender (1947) report x-ray effects on isolated DNA but conclude that the nucleic acid is not destroyed, since no chemical property is altered. Both investigations reveal a loss of

streaming birefringence and a great drop in viscosity of the DNA after x-ray treatment. Since both the magnitude of relative viscosity and the intensity of flow birefringence are indicative of the degree of molecular asymmetry, the above changes in these properties probably represent a depolymerization or rearrangement of the molecular particles.

Direct observations with a microscope of Feulgen-stained tissue show that nuclei which were given a dose of 4000 r or more are less intensively stained than non-irradiated nuclei. However, when nuclear measurements and light absorption readings are made, it is evident that there is not a significant decrease of DNA desoxypentose. The apparent visual loss of Feulgen stainability would seem to be the result of nuclear swelling, that is, a change in the distribution of the DNA in the nucleus. With microscopic observations the intensity and color of irradiated and control nuclei appear to be the same in tissue stained with methyl green, but photometric measurements disclose a highly significant loss in stainability after radiation.

We are very appreciative of the counsel and encouragement given us by Dr. J. Gordon Carlson of The University of Tennessee and Dr. Mary Esther Gaulden of the Oak Ridge National Laboratory. We gratefully acknowledge our indebtedness to Mr. Jack Moshman of the Oak Ridge National Laboratory Mathematics Panel for the statistical treatment of our data.

Summary

1. Cytological and cytochemical studies were made on embryonic nuclei of the grasshopper, *Chortophaga viridifasciata*, after x-ray doses of 4000, 10,000, and 12,500 r. The changes induced were photometrically measured by using (1) the Feulgen reaction to determine relative changes in the DNA desoxypentose, and (2) the methyl green stain to indicate the degree of polymerization of the nucleic acid.

2. X-radiation was found to cause swelling of the nuclei. When correction was made for this, the Feulgen-stained nuclei showed no significant loss of DNA after irradiation, but the nuclei stained with methyl green disclosed a highly significant loss of stainability. This is interpreted to indicate that x-rays do not destroy the DNA but induce depolymerization of the nucleic acid.

3. This study has shown that it is not safe to make quantitative estimates of DNA (stained by Feulgen or methyl green) from microscopic observations and photographs. The visually apparent loss of Feulgen-stainability after irradiation is probably not due to a decrease in the DNA desoxypentose but rather to the increased dispersion of the DNA in nuclei that have undergone x-ray-induced enlargement.

LITERATURE CITED

Baker, J. R., 1942. Some aspects of cytological technique. Cytology and cell physiology, edited by G. Bourne. Oxford University Press, London. Pp. 21–24.

Caspersson, T., 1932. Die quantitative Bestimmung von Thymonucleinsäure mittels fuchsinschwefliger Säure. *Biochem. Zeitschr.*, **235**: 97.

Caspersson, T., 1936. Über den chemischen Aufbau der Strukturen des Zellkerns. *Skand. Arch. Physiol., Suppl. 8*, **73**: 1–151.

Caspersson, T., 1940. Die Eiweissverteilung in den Strukturen des Zellkerns. *Chromosoma*, **1**: 562–604.

Caspersson, T., 1941. Studien über den Eiweissumsatz der Zelle. *Naturwissenschaften*, **29**: 33–43.

Caspersson, T., and J. Schultz, 1938. Nucleic acid metabolism of the chromosomes in relation to gene reproduction. *Nature*, **142**: 294–295.

Caspersson, T., and J. Schultz, 1940. Ribonucleic acid in both nucleus and cytoplasm, and the function of the nucleolus. *Proc. Nat. Acad. Sci.*, **26**: 507–515.

Catcheside, D. G., and B. Holmes, 1947. The action of enzymes on chromosomes. Symposia of the Society for Experimental Biology, No. 1. Nucleic acid. Cambridge University Press, London. Pp. 225–231.

Coleman, L. C., 1938. Preparation of leucobasic fuchsin for use in the Feulgen reaction. *Stain Technol.*, **13**: 123–124.

Darlington, C. D., and L. F. La Cour, 1945. Chromosome breakage and the nucleic acid cycle. *J. Genetics*, **46**: 180–267.

Darlington, C. D., and L. F. La Cour, 1947. The handling of chromosomes. George Allen and University Ltd., London. Pp. 117, 129.

Darlington, C. D., and K. Mather, 1949. The elements of genetics. Macmillan Co., New York. Pp. 145–147.

Di Stefano, H. S., 1948. A cytochemical study of the Feulgen nucleal reaction. *Chromosoma*, **3**: 282–301.

Ely, J. O., and M. H. Ross, 1949. Nucleic acids and the Feulgen reaction. *Anat. Record*, **104**: 103–119.

Failla, G., 1940. Some aspects of the biological action of ionizing radiations. *Amer. J. Roentgenol.*, **44**: 649–664.

Glücksmann, A., 1941. Quantitative examination of human biopsy material taken from irradiated carcinomata. *Brit. J. Radiol.*, **14**: 187.

Hillary, B. B., 1940. Uses of the Feulgen reaction in cytology. *Bot. Gaz.*, **102**: 225–235.

Holweck, F., and A. Lacassagne, 1930. Action sur les levures des rayons Xmous (K du fer). *Compt. Rend. Soc. Biol., Paris*, **103**: 60–62.

Koller, P. C., 1947. The experimental modification of nucleic acid systems in the cell. Symposia of the Society for Experimental Biology, No. 1. Nucleic acid. Cambridge University Press, London. Pp. 270–288.

Kurnick, N. B., 1950a. The quantitative estimation of desoxyribosenucleic acid based on methyl green staining. *Exp. Cell Research*, **1**: 151–158.

Kurnick, N. B., 1950b. Methyl-green pyronin I. Basis of selective staining of nucleic acids. *J. Gen. Physiol.*, **33**: 243–264.

Kurnick, N. B., 1950c. The determination of desoxyribonuclease activity by methyl-green; application to serum. *Arch. Biochem.*, **29**: 41–53.

Lea, D. E., 1947. Action of radiations on living cells. Macmillan Co., New York. Pp. 300–301.

Lea, D. E., R. B. Haines and C. A. Coulson, 1937. The action of radiations on bacteria. III. Gamma rays on growing and on non-proliferating bacteria. *Proc. Roy. Soc. London, Ser. B*, **123**: 1–21.

Leuchtenberger, C., 1950. A cytochemical study of pycnotic nuclear degeneration. *Chromosoma*, **3**: 449–473.

Lumb, E. S., 1950. Cytochemical reactions of nucleic acids. *Quart. Rev. Biol.*, **25**: 278–291.

Mosteller, F., 1948. A k-sample slippage test for an extreme population. *Ann. Math. Statistics*, **19**: 58–65.

Mottram, J. C., 1927. Early change in nucleus of cells of tumors exposed to beta rays. *Brit. J. Radiol.*, **32**: 61–64.

Mottram, J. C., 1933. Changes in the non-dividing nucleus following gamma radiation. *J. Roy. Microscop. Soc.*, **53**: 213–219.

Pollister, A. W., and C. Leuchtenberger, 1949. The nature of the specificity of methyl green for chromatin. *Proc. Nat. Acad. Sci.*, **35**: 111–116.

Pollister, A. W., and H. Ris, 1947. Nucleoprotein determination in cytological preparations. *Cold Spring Harbor Symp. on Quant. Biol.*, **12**: 147–157.

Ris, H., and A. E. Mirsky, 1949. Quantitative cytochemical determination of DNA with the Feulgen nucleal reaction. *J. Gen. Physiol.*, **33**: 125–146.

ROBERTSON, M., 1935. On the reduction in the multiplication of a protozoon (*Bobo caudatus*) caused by the exposure to gamma ray irradiation with a study of the sensitive period in the life of the cell. *Brit. J. Radiol.*, **8**: 502–525.

SCHRADER, F., AND C. LEUCHTENBERGER, 1950. A cytochemical analysis of the functional interrelations of various cell structures in *Arvelius albopunctatus* (De Geer). *Exp. Cell Research*, **1**: 421–452.

SERRA, J. A., 1949. The parallelism between the chemical and the morphological changes in the chromosomes during mitosis and meiosis. *Exp. Cell Research*, Suppl. 1, 111–122.

SPARROW, A. H., AND F. M. ROSENFELD, 1946. X-ray induced depolymerization of thymonucleohistone and of sodium thymonucleate. *Science*, **104**: 245–246.

STOWELL, R. E., 1945. Feulgen reaction for thymonucleic acid. *Stain Technol.*, **20**: 45–58.

STOWELL, R. E., 1946. The specificity of the Feulgen reaction for thymonucleic acid. *Stain Technol.*, **21**: 137–148.

SVEDBERG, T., AND S. BROHULT, 1939. Splitting of protein molecules by ultra-violet light and alpha rays. *Nature*, **143**: 938–939.

SWIFT, H. H., 1950. The desoxyribose nucleic acid content of animal nuclei. *Physiol. Zool.*, **23**: 169–198.

TAYLOR, B., J. P. GREENSTEIN AND A. HOLLAENDER, 1947. Effects of X-radiation on thymus nucleic acid. *Science*, **105**: 263–264.

WYCKOFF, R. W. G., A. H. EBELING AND A. L. TER LOUW, 1932. A comparison between the ultra-violet microscopy and the Feulgen staining of certain cells. *J. Morphol.*, **53**: 189–199.